

1 48. A method for measuring an activity of an intracellular chemical reaction of a  
2 single species of a molecule of a selected cell or cellular component thereof, said  
3 method comprising:  
4 (a) controllably selecting at least one of a plurality of cells or cellular  
5 component thereof, in a medium;  
6 (b) selecting a substrate specific to said single species of said molecule;  
7 (c) providing a label for said substrate;  
8 (d) inducing or permitting the catalysis of a reaction between said single  
9 species of said molecule and said substrate, said reaction producing altered  
10 substrate molecules and including attachment of said label to said altered  
11 substrate;  
12 (e) disrupting said at least one selected cell or cellular component thereof with  
13 a laser generated shock wave in said medium in close proximity to said  
14 selected cell or component thereof to terminate said reaction;  
15 (f) collecting said substrate, altered substrate, or portions thereof; for  
16 (g) analyzing said collected substrate in an analysis device; and  
17 (h) determining activity of said chemical reaction from a comparison of  
18 detected altered substrate molecules with detected substrate molecules.

1 49. The method of claim 48 further comprising simultaneously mixing said contents  
2 of said lysed cell or cellular component thereof with said substrate when collecting at  
3 least a portion of said substrate.

1 50. The method of claim 48 wherein said portion of said substrate of said cell or  
2 cellular component thereof is collected within 33 msec or less of lysis of said cell.

1 51. The method of claim 48 wherein said portion of said substrate of said cell or  
2 cellular component thereof is collected within a 1 - 10 microseconds of lysis of said cell.

1 52. The method of claim 48 wherein controllably selecting at least one of a plurality  
2 of cells or cellular component thereof comprises identifying and relatively positioning  
3 said selected cell or cellular component thereof.

1 53. The method of claim 48 wherein collecting at least a portion of said substrate of  
2 said cell or cellular component thereof comprises stopping a reaction of biochemical  
3 reactants disrupted from said selected cell or cellular component thereof to permit  
4 subsequent analysis of said biochemical reactants in the state which existed  
5 approximately at the time of disruption.

1 54. The method of claim 52 where relatively positioning said selected cell or cellular  
2 component thereof in said medium comprises adhering said cell or cellular component  
3 thereof to a substrate disposed at least adjacent to said medium.

1 55. The method of claim 52 where said cell or cellular component is free floating and  
2 where controllably positioning said selected cell or cellular component thereof in said  
3 medium comprises temporarily holding said cell or cellular component thereof in a  
4 position in said medium by laser microbeam optical tweezers.

1 56. The method of claim 52 where said cell or cellular component is free floating and  
2 where controllably positioning said selected cell or cellular component thereof in said  
3 medium comprises temporarily holding said cell or cellular component thereof in a  
4 position in said medium by adhesion of a mechanical micromanipulator-held pipette or  
5 other device to said cell or cellular component thereof.

1 57. The method of claim 52 where said cell or cellular component is free floating and  
2 where relatively positioning said selected cell or cellular component thereof in said  
3 medium comprises positioning said cell or cellular component thereof in a confined  
4 enclosure.

1 58. The method of claim 57 where said cell or cellular component is free floating and  
2 where positioning said cell or cellular component thereof in said confined enclosure  
3 comprises positioning said cell or cellular component thereof in an inlet to an analysis  
4 device.

1 59. The method of claim 48 where collecting at least a portion of said substrate in  
2 said analysis device collects said cell or cellular component thereof in an electrophoretic  
3 column or channel.

1 60. The method of claim 49 where collecting at least a portion of said substrate in  
2 said analysis device collects said cell or cellular component thereof in an electrophoretic  
3 column or channel.

1 61. The method of claim 48 where producing a laser generated shock wave in close  
2 proximity to said selected cell or cellular component comprises focusing a pulsed laser  
3 beam at a position proximate to said cell or cellular component thereof without focusing  
4 on said cell or cellular component thereof, and generating said shock wave.

1 62. The method of claim 48 where producing a laser generated shock wave in close  
2 proximity to said selected cell or cellular component thereof comprises focusing a  
3 pulsed laser beam directly in or on said cell or cellular component thereof, and  
4 generating said shock wave.

1 63. The method of claim 62, further comprising defining an opening in said cell of  
2 cellular component thereof to lyse only cytoplasmic contents therefrom by said step of

3 focusing a pulsed laser beam directly in or on said cell or cellular component thereof to  
4 lyse said cell or cellular component thereof.

1 64. The method of claim 48 where collecting at least a portion of said substrate of  
2 said lysed cell or cellular component thereof in said analysis device is by means of fluid  
3 flow of said medium.

1 65. The method of claim 64 where collecting at least a portion of said substrate of  
2 said lysed cell or cellular component thereof in said analysis device is by means of  
3 siphon fluid flow of said medium.

1 66. The method of claim 48 where collecting at least a portion of said substrate of  
2 said lysed cell or cellular component thereof in said analysis device is by means of  
3 electrophoresis through said medium.

1 67. The method of claim 64 where collecting at least a portion of said substrate of  
2 said lysed cell or cellular component thereof in said analysis device is by means of force  
3 from said shock wave imparted to said substrate.

1 68. The method of claim 64 where collecting at least a portion of said substrate of  
2 said lysed cell or cellular component thereof in said analysis device is by means of  
3 electroosmotic fluid flow.

1 69. The method of claim 48 wherein producing a laser generated shock wave in  
2 close proximity to said selected cell in said medium is performed at an energy density  
3 level just sufficient to split open said selected cell so that substantially all of said  
4 contents of said lysed cell or cellular component thereof and said substrate remain  
5 proximate to said lysed cell or cellular component thereof.

1 70. The method of claim 48 where analyzing said collected substrate comprises  
2 analyzing said collected cell contents by means of laser induced fluorescence.

1 71. The method of claim 48 further comprising utilizing said collected substrate.

1 72. The method of claim 48 where collecting said portion of said substrate comprises  
2 collecting by means of a microlumen of a capillary of a micropipette.

1 73. The method of claim 48 where collecting said portion of said substrate comprises  
2 collecting by means a microlumen of a microfabricated channel.

1 74. The method of claim 48 where collecting said portion of said substrate comprises  
2 collecting by means of a microlumen, and further comprises aspirating said portion of  
3 said substrate into said microlumen.

1 75. The method of claim 74 where aspirating said portion of said substrate into said  
2 microlumen comprises aspirating said portion of said substrate into a capillary of a  
3 micropipette.

1 76. The method of claim 74 where aspirating said portion of said substrate into said  
2 microlumen comprises aspirating said portion of said substrate into a microfabricated  
3 channel.

1 77. The method of claim 48 where collecting said portion of said substrate comprises  
2 collecting by means of a microlumen, and comprises collecting said portion of said  
3 substrate within one second of producing a laser generated shock wave to lyse said  
4 cell.

1 78. The method of claim 48 where collecting said portion of said substrate comprises  
2 collecting by means a microlumen, and comprises collecting said accessible substrate  
3 within 33 msec of producing a laser generated shock wave to lyse said cell.

1 79. The method of claim 48 where collecting said portion of said substrate  
2 comprises collecting by means of a microlumen, and comprises collecting said portion  
3 of said substrate within 10 microseconds of producing a laser generated shock wave to  
4 lyse said cell.

1 80. The method of claim 48 where collecting said portion of said substrate comprises  
2 collecting by means of a microlumen, and comprises collecting said portion of said  
3 substrate within 1 microsecond of producing a laser generated shock wave to lyse said  
4 cell.

1 81. The method of claim 48 where said step of analyzing further comprises analyzing  
2 said substrate after lysis by an analysis device, wherein said substrate has no  
3 substantial difference in form between the condition of said substrate before and after  
4 lysing.

1 82. A method for measuring the intracellular chemical activity of a single species of a  
2 molecule in a cell comprising:  
3 providing a substrate molecule specific to said single species;  
4 disposing said substrate molecule into said cell for allowing in vivo reaction  
5 therein with said single species of said molecule, said in vivo reaction defined as  
6 substrate turnover;  
7 reacting said substrate with said single species of said molecule in said cell to  
8 cause a change in a chemical characteristic of said substrate by said substrate turnover  
9 to provide an intracellular reporter of said intracellular chemical activity in said cell; and  
10 disrupting said step of reacting and detecting said substrate turnover by lysis of  
11 said cell and by measurement of said substrate turnover after lysis before any  
12 substantial alteration of products of said substrate turnover has occurred.



1 83. An apparatus for lysing and collecting the contents of one of a plurality of cells or  
2 cellular component thereof in a medium in order to measure an activity of an  
3 intracellular chemical reaction of a single species of molecules in said cell or cellular  
4 component thereof, said apparatus comprising:

5 a cell selector to controllably select at least one of said cells or cellular  
6 component thereof;

7 a laser for generating a shock wave pulse to lyse said at least one selected cell  
8 at a location in said medium in sufficiently close proximity to said selected cell such that  
9 said cell is lysed to permit at least a portion of the contents of said selected cell  
10 including a substrate specific to said single species of molecules to be accessible to  
11 said medium;

12 a collector to capture at least a portion of said substrate in a form having no  
13 substantial difference between the condition of said substrate before and after lysis.

1 84. The apparatus of claim 83 further comprising an analysis device for analyzing  
2 said substrate in a form having no substantial difference between the condition of said  
3 substrate before and after lysis.

1 85. The apparatus of claim 84 wherein said collector delivers at least said portion of  
2 said substrate from said lysed cell to said analysis device within one second of lysis of  
3 said cell.

1 86. The apparatus of claim 84 wherein said collector delivers at least said portion of  
2 said substrate from said lysed cell to said analysis device within 33 msec of lysis of said  
3 cell.

1 87. The apparatus of claim 84 wherein said collector delivers at least said portion of  
2 said substrate from said lysed cell to said analysis device within 1 - 10 microseconds of  
3 lysis of said cell.

1 88. The apparatus of claim 84 wherein said collector delivers at least said portion of  
2 said substrate from said lysed cell to said analysis device within 1 microseconds of lysis  
3 of said cell.

1 89. The apparatus of claim 84 wherein said analysis device comprises a means for  
2 performing polymerase chain reactions on said contents, a means of separating  
3 products of said polymerase chain reactions, and a means for detecting separated  
4 molecules.

1 90. (once amended) The apparatus of claim 84 wherein said collector delivers said  
2 portion by flowing said medium past said cell during lysis into said analysis device.

1 91. The apparatus of claim 84 wherein said collector delivers said portion by  
2 providing electrophoresis in said medium between said analysis device and said cell  
3 during lysis thereof.

1 92. The apparatus of claim 84 wherein said collector delivers said portion by  
2 providing an electroosmotic flow in said medium between said analysis device and said  
3 cell during lysis thereof.

1 93. The apparatus of claim 84 wherein said collector delivers said portion by  
2 providing force from a shock wave in said medium to move said cell into said analysis  
3 device during lysis of said cell.

1 94. An apparatus for measuring the intracellular chemical activity of a single species  
2 of molecules in a cell comprising:  
3 first means for disposing a substrate molecule specific to said single species of  
4 molecules into said cell for allowing in vivo reaction therein, said in vivo reaction defined  
5 as substrate turnover, wherein said substrate molecules react with said single species  
6 of molecules in said cell to cause a change in a chemical characteristic of said substrate  
7 resulting in said substrate turnover to provide an intracellular reporter of said  
8 intracellular chemical activity in said cell; and

9           second means for disrupting said reaction and for detecting said substrate  
10 turnover by lysis of said cell and by measurement of said substrate turnover after lysis  
11 before any substantial alteration of products of said substrate turnover has occurred.

1   95.           A method for measuring the activity of an intracellular chemical reaction  
2 in a cell, a portion of a cell, or a group of cells comprising:  
3           selecting a target molecule of a single species;  
4           providing substrate molecules corresponding to said target molecule of said  
5 single species, said substrate molecules containing a label, the labeled substrate  
6 molecules corresponding to the chemical reaction whose activity is to be measured;  
7           disposing said substrate molecules within said cell, portion of said cell, or said  
8 group of cells;  
9           allowing said substrate molecules within the cell, portion of said cell, or said  
10 group of cells to take part in the chemical reaction to produce altered substrate  
11 molecules;  
12           liberating said substrate molecules and said altered substrate molecules from the  
13 cell, portion of said cell, or said group of cells;  
14           spatially separating said substrate molecules and said altered substrate  
15 molecules;  
16           detecting the label to identify the substrate molecules and/or the altered  
17 substrate molecules; and  
18           determining activity of said chemical reaction from a comparison of detected  
19 altered substrate molecules with detected substrate molecules.

1 96. The method of claim 95, wherein said cell, portion of said cell, or said group of cells  
2 is selected from among mammalian cells that have a volume of approximately 1 nl.

1 97. A method for measuring activity of a chemical reaction in a minute  
2 volume of 100 picoliters or less comprising:  
3 selecting a target molecule of a single species;  
4 providing substrate molecules corresponding to said target molecule of said  
5 single species and containing a label;  
6 disposing said substrate molecule into said minute volume where a chemical  
7 reaction occurs producing altered substrate molecules within said minute volume;  
8 terminating said chemical reaction;  
9 detecting the label to identify the substrate molecules and the altered substrate  
10 molecules to determine activity of the chemical reaction.

1 98. The method of claim 96, wherein said label is a fluorescent label, said step  
2 of detecting further comprising:  
3 separating said substrate molecules and said altered substrate molecules by  
4 capillary electrophoresis; and  
5 quantifying fluorescence of said substrate molecules and said altered substrate  
6 molecules.

1 99. An apparatus for measuring an activity of a chemical reaction of a single  
2 species of intracellular molecules comprising:

3 means for disposing labeled substrate molecules into a cell, portion of said cell,  
4 or a group of cells which undergo a chemical reaction to form labeled and altered  
5 substrate molecules therein;

6 means for liberating said substrate and altered substrate molecules from said  
7 cell, portion of said cell, or said group of cells;

8 means for separating said substrate and altered substrate molecules from each  
9 other;

10 means for detecting said substrate molecules and said altered substrate  
11 molecules from said cell, portion of said cell, or said group of cells before any  
12 substantial alteration of said substrate molecules and said altered substrate molecules  
13 has occurred; and

14 means for isolating and detecting a reaction of said single species of intracellular  
15 molecules.

1 100. An apparatus for measuring an activity of a chemical reaction of a single  
2 species of molecules in a minute volume of the order of 100 picoliter or less comprising:

3 means for disposing substrate molecules corresponding to said single species of  
4 molecules and having a label into said minute volume for a chemical reaction to occur  
5 producing altered substrate molecules;

6 means for separating the substrate molecules and the altered substrate  
7 molecules; and  
8 means for detecting the label to identify the substrate molecules and the altered  
9 substrate molecules to determine activity of the chemical reaction.

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1 101. An apparatus for measuring an activity of an intracellular chemical  
2 reaction of a single species of molecules in a cell, portion of said cell, or said group of  
3 cells in which labeled substrate molecules corresponding to said single species of  
4 molecules have been disposed to allow for an in vivo reaction wherein labeled and  
5 altered substrate molecules are formed, comprising:  
6 an interrupter, which interrupter stops the chemical reactions immediately prior to  
7 detection and sampling;  
8 a detector of said labeled substrate molecules and said labeled altered substrate  
9 molecules;  
10 a sampling device communicating with said detector, which sampling device  
11 extracts said substrate and altered substrate molecules from said cell, portion of said  
12 cell, or group of said cells and which sampling device collects and transfers said  
13 substrate and altered substrate molecules into said detector before any substantial  
14 alteration occurs.

1 102. (once amended) An apparatus for measuring an activity of an intracellular  
2 chemical reaction of a single species of molecules in a mammalian cell in which labeled  
3 substrate molecules corresponding to said single species of molecules have been  
4 disposed to allow for an in vivo reaction, wherein labeled altered substrate molecules  
5 are formed, comprising:

6 means for holding the mammalian cell;

7 an electrophoresis reservoir contiguous to but not in fluidic contact with

8 said means for holding;

9 a sharpened electrophoresis capillary for puncturing the mammalian cell to

10 remove a cellular sample;

11 means for moving said electrophoresis capillary to puncture said

12 mammalian cell;

13 means for rapidly transitioning said capillary into contact with said

14 electrophoresis reservoir after removing said cellular sample so that

15 electrophoresis of said cellular sample through said capillary will

16 commence; and

17 a detector for detecting said labeled substrate molecules and said labeled

18 altered substrate molecules during or following electrophoresis.

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